Objection to Claims 8 and 10

Claims 8 and 10 have been objected to for reciting or encompassing non-elected inventions.

This objection is not understood, as Claims 8 and 10 are clearly within the claims of the Group I invention (Claims 6-10) as set forth in the Office Action of 5 August 2001 outlining the groups of claims requiring restriction. The Group I claims were elected in a Reply to Restriction Requirement mailed to the United States Patent and Trademark Office on 5 July 2001 and in a second Reply to Restriction Requirement specifying an election of species, mailed to the United States Patent and Trademark Office on 20 August 2001. In the Reply to Restriction Requirement of 20 August 2001, Claims 7-10 were those recited by Applicants as being readable on the elected species SEQ ID NO:9. Clarification of the objection is respectfully requested.

Rejection of Claim 12 Under 35 U.S.C. § 112, Second Paragraph

Claim 12 is rejected under 35 U.S.C. § 112, second paragraph, as "being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention."

Claim 12 has been withdrawn from consideration. With the amendments to the claims made in the Preliminary Amendment mailed to the United States Patent and Trademark Office on 5 July 2001, no claim recites the term "pseudo-pseudo-ligand." Claim 9 recites the term "pseudo-ligand" with proper antecedent basis in Claim 8.

Rejection of Claims 6-10 Under 35 U.S.C. § 112, First Paragraph

Claims 6-10 have been rejected under 35 U.S.C. § 112, first paragraph, because it is said that "the subject matter was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." The Examiner further states that "[t]he specification is not enabling for the limitations of the claims wherein apoptosis is prevented in epidermal keratinocytes as a method of causing hair growth."

The experiment having results depicted in the bar graph of Figure 12B illustrated that the peptide having amino acid sequence SEQ ID NO:9 prevented the loss of NIH-3T3 cells in

culture. The results in Figures 12A and 12C show that other KGA peptides also prevented loss of NIH-3T3 cells. Also see the description in Example 18, page 48, line 7 to page 49, line 2. Other evidence that the KGA amino acid sequence is involved in binding to $p75^{NTR}$ is found in Example 17, on page 47, line 27 to page 48, line 6, and in Figure 11. From the experiment described in Example 10, page 41, lines 7-30, and in Figures 9A and 9B, it can be concluded that binding of nerve growth factor increases survival of keratinocytes irradiated with ultraviolet light.

Instruction on practicing the methods of the claims can be found on page 29, line 4 to page 31, line 15. One of ordinary skill in the art, using this description, would be able to determine dosages and methods of administering the described p75 nerve growth factor ligands that are effective in preventing hair loss. Topical administration of such non-toxic ligands, as described on page 30, lines 3-17, is particularly easily tested, with results on hair growth easily observed without any complicated assay.

The Examiner states a conclusion, that is not stated in, and cannot be derived from the recent literature cited, that "apoptosis of cultured keratinocytes caused by irradiation with UV light is a poor model of hair loss in a mammal."

Applicants demonstrate, with the Declaration of Barbara A. Gilchrest, M.D. Under 37 U.S.C. § 1.132 being filed concurrently, that cultured cells are an appropriate model for the behavior of hair follicles, and that conclusions about hair growth can be made on the basis of experiments performed on cultured cells.

Form PTO-948

An Attachment for Form PTO-948 was received with the Office Action dated 6 November 2002. Because no Form PTO-948 detailing informalities to be corrected in the drawings was included with the Attachment, it is assumed that the Attachment was sent in error.

Paragraph Relating to Final Action

The Office Action includes, on pages 7-8, a paragraph pertaining to the "shortened statutory period for reply to this final action." It is assumed that this paragraph was included in error, because page 1 of the Office Action specifically indicates that the action is non-final.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 39, lines 1 through 11 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Purified phosphorothioate oligonucleotides were purchased from Quality Controlled Biochemicals, Inc. (Hopkinton, MA). [19 mer] 19-mer oligonucleotides were designed based on the published human BCL-2 sequence (Tsujimoto, Y. and Croce, C.M., Proc. Natl. Acad. Sci. USA, 83:5214-5218 (1986)). The sequence chosen was directed against the 5' end of the coding region starting 4 bases before the methionine initiation site. Nonsense oligonucleotides were used as control. Sequences used (all written 5'→3'): Antisense CCCAGCGTGCGCCATCCTT (SEQ ID NO: 7); Nonsense CTCCCACTCGTCATTCGAC (SEQ ID NO: 8).

Replace the paragraph at page 39, line 12 through page 40, line 10 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

MM4 cells were maintained in 60 mm diameter tissue culture dishes in 55.3% DME, 27.6% L15, 15% FBS, 1% nonessential amino acids (GIBCO BRL), 2 mM glutamine and 10 μg/ml insulin. Near confluent cells were UV irradiated with 10 mJ/cm^2. Immediately after irradiation cells were incubated with 10 uM antisense or nonsense BCL-2 oligonucleotides in suspension at 37°C for 30 minutes. Then cells were plated in tissue culture dishes in the presence or absence of NGF (50 ng/ml). Cells were supplemented with fresh oligonucleotides every 12 hours. Cell yield and BCL-2 level were determined 48 hours after irradiation. Cell yield was determined by counting cells in at least three representative field per each condition. Figure 8 shows the results of a Western blot demonstrating that in the presence of antisense BCL-2 oligonucleotides BCL-2 levels are almost undetectable. Cell yields of UV irradiated cultures supplemented with NGF and nonsense oligonucleotides (white bar) are significantly higher as compared to nonsense supplemented cultures

provided with diluent alone (dotted bar) ($p<0.007$, ANOVA). Cell yields of NGF supplemented cultures treated with BCL-2 antisense oligonucleotides (black bar) are significantly lower than NGF supplemented cultures provided with nonsense oligonucleotides (white bar) demonstrating complete abrogation of NGF effect on the cells ($p<0.003$, ANOVA). In diluent supplemented culture yields of nonsense treated cells (dotted bar) were significantly higher than antisense treated cells (dashed bar) ($p<0.004$, ANOVA). Morphologic appearance of MM4 cells confirmed the numerical cell yield data. This experiment demonstrated that BCL-2 protein is required for melanocytic survival after UV irradiation and that NGF affects melanocytic cell survival by upregulating their BCL-2 level.

Replace the paragraph at page 40, line 13 through page 41, line 6 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Cells were washed with cold PBS and disrupted in lysis buffer [pH8] pH 8 (10 mM [tris] Tris, 150 mM [NaCl] NaCl, 0.1 mM EDTA, 1% SDS, 200 µg/ml proteinase K). After 15 hour incubation at 37°C, samples were extracted twice with phenol plus chloroform (1:1, V/V) and precipitated overnight with ethanol (2.5 X volume) and 3 M sodium acetate (1/10 x volume). The DNA was then digested with DNAse free ribonuclease (10 µg/ml) for one hour at 37°C, separated on 1% agarose gel and stained with ethidium bromide. The data show that DNA fragmentation, characteristic of apoptotic cell death, occurs in UV-irradiated but not sham irradiated keratinocytes.

Replace the paragraph at page 41, lines 9 through 30 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Keratinocytes were UV-irradiated as in Example 9. After irradiation cells were placed in fresh keratinocyte medium containing 50 ng/ml NGF or diluent alone. DNA fragmentation was determined as in Example 9. Figure 9A shows that UV irradiated keratinocytes supplemented with diluent alone (-) display the characteristic DNA fragmentation, while DNA of UV irradiated cells supplemented with NGF (+) is not fragmented. The standard (STD) is 100 bp DNA ladder

(Gibco/BRL). Keratinocyte yield determined daily for 5 days as shown in Figure 9B demonstrates that within 24 hours there is a 50% decrease in cell yield in cultures provided with diluent alone but [on] only 30% decreases in cultures provided with NGF. UV irradiated keratinocytes were growth arrested as expected. However, cell yields of keratinocytes maintained in NGF supplemented medium increased by 40% within the 5 days of the experiment, suggesting that NGF is a mitogen for keratinocytes as well as a survival factor. This experiment demonstrates that, similar to melanocytes, NGF is a survival factor for keratinocytes. Furthermore, the experiment suggests that NGF might be a mitogen for keratinocytes as well.

Replace the paragraph at page 46, lines 27 through 29 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

$p75^{NTR}$-NIH 3T3 cells were [maintain] maintained in DMEM supplemented with 10% FBS in the presence of penicillin (45 ng/ml), streptomycin (68 ng/ml), and hygromycin B (17.5 ng/ml).

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

6. (Amended) A method of [inducing] preventing hair [growth] loss in a [vertebrate] mammal comprising inhibiting apoptosis in epidermal keratinocytes.